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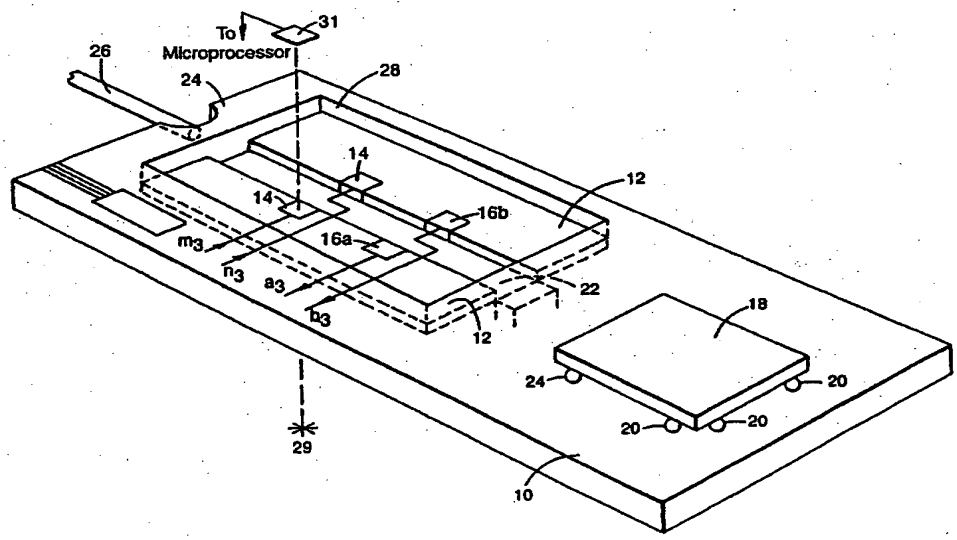
(54) Title: APPARATUS FOR, AND METHOD OF, INTRODUCING A SUBSTANCE INTO AN OBJECT




(57) Abstract

The invention relates to an apparatus for and method of introducing a substance into an object, particularly into a cell or cellular material. In a preferred arrangement apparatus receives the cell and preferably locates it between first and second electrodes and applies a voltage pulse to cause a disruption in the cell wall. This causes the cell to become permeable. The substance may then be introduced, for example under a fluid pressure. Cells may then be inspected and sorted into transfected and non–transfected types. This may be achieved automatically, for example by using electrophoresis.

# APPARATUS FOR, AND METHOD OF, INTRODUCING A SUBSTANCE INTO AN OBJECT

This invention relates to an apparatus for, and method of, introducing a substance into an object.

More particularly, but not exclusively, the invention is capable of introducing substances into small objects, such as for example, cellular material or cells. The substance introduced may be a transfecting agent such as for example: a chemical, molecule, protein, virus, prion or DNA material.

The invention will hereinafter be described with reference to cells for clarity and conciseness. However, it will be appreciated that the invention is not limited to this application and that within the specification the terms cells and objects are interchangeable.

Previously material has been introduced into cells by way of a syringe-like device. These syringe-like devices have to be very small, typically less than a few micrometres in diameter, so that they can penetrate the cell wall. Partly due to this reason, it has been very difficult to manufacture sufficiently robust syringe-like devices.

The present invention arose to overcome this and other problems associated with syringe-like devices.

characteristics. Consequently the potential difference may be applied with greater precision.

A proximity detector is advantageously included, so that when an object is in the predetermined part of the electric field, the voltage pulse is applied automatically. Processing means, including electronic logic, may be used to improve and enhance this process.

Preferably there is provided a plurality of the aforementioned apparatuses arranged in an array. An advantage of such an array is that many objects may be acted upon in parallel. This increases throughput. For the sake of simplicity, the process of acting on objects so as to introduce a substance is hereinafter referred to as transfection. Transfection includes the process of introducing a substance into an object.

An array of apparatuses may be formed on a semiconductor substrate, such as for example, silicon or germanium. Proximity detectors, electrodes and processing means may be included on the substrate, for example, in a different layer of an integrated semiconductor structure.

In a particularly preferred embodiment DNA is introduced into living cells by rupturing or forming a discontinuity in the cell wall by the process of electroporation. Cells are supported in a fluid and are able to move with respect to the electrodes.

The channel is preferably narrow, for example, between 1 and 5 times the diameter of the cells (which may typically be around 5 – 10 μm) to be electroporated. Such narrow channels are advantageous in electroporation, as the electroporation voltage may be applied across the cell per se, rather than across the cell and the supporting fluid. This enables the electric field experienced by the cell to be controlled precisely. In an alternative embodiment the channel may even be narrower than the diameter of the cell in its relaxed state. In this embodiment cells deform and flow along the channel and are in closer contact with the walls.

Alternatively the channel or well may be relatively wide except for a constriction at a region at which transfection occurs, the constriction, and/or electroporation electrodes may be designed so that pores, opened in the cell membrane, to allow transfection, are preferentially oriented at a source of a transfecting agent.

In a microfabricated device electronic logic may be used to control the amplitude of the electroporation voltage pulse or sequence of pulses. The logic circuitry may be integrated within a semiconducting substrate, for example using CMOS, DMOS or bi-polar components, fabricated in a convenient process sequence. Preferably the substrate also forms a support for microfabricated channels. Post-processing techniques can be used during manufacture of the substrate to interconnect electronic components to electrode flow channel(s).

Integration of a moderately high-voltage (typically 5-25 volts) switch device (used to control the electroporation voltage) is especially advantageous as the minimal electrical

facilitates connection of processing or control circuitry to external processors, such as a microprocessor for closed loop flow control and/or electroporation pulse application. Closed loop flow control is used to increase/decrease the rate of cell occupancy of wells.

Preferably sensing means is provided which operates in conjunction with an electrode and a common ground, or a pair of electrodes, to interrogate a channel or well for determining the presence of a cell.

Preferably control means controls the instant of electroporation pulse timing, in collaboration with the detection of a cell in the well or channel. For example, a microcontroller, timer or state-machine may be integrated and used to control the instant of application and/or amplitude of an electroporation pulse, in response to a signal indicating the presence of a cell.

Preferably means is provided to determine the state or condition of cells following transfection, indicating whether a cell is unaffected, transfected or damaged. For example apparatus as described in published International Patent Application No WO-A-9402846 (BTG) may be utilised for this purpose. Thus it is possible to characterise the cell, at a locus or loci both prior to and following an attempt at transfection, and to determine the success or otherwise by the difference in the cell's response rather than by an absolute calibration.

Subsequent to transfection of cells, there is preferably provided sorting means to direct cells into a collection flow or to a waste flow depending on the result of transfection. Such sorting may be achieved by a number of methods and include: electrophoresis, dielectrophoresis, "optical tweezers", and/or directed pressure pulses.

Means may also be provided to destroy cells which have not been transfected in the desired manner. Such destruction may be achieved by killing the cell(s) while leaving it/them essentially physically intact or by physically disrupting the cell(s). Preferred means of achieving this include: electrical disruption of the cell, essentially by overly vigorous electroporation, the introduction of a cell lysing agent; or rapid local heating of the cell or fluid medium in the vicinity of the cell. A micro-heating element or directed, pulsed infra red radiator or laser may be used for this purpose.

In cases where the electroporation apparatus has electrical connections, routed about or around it, for example in a highly integrated active substrate, it may be desirable to provide electrical guard bands suitably disposed around portions of a fluid handling structure so that any electric field applied to the fluid is reduced sufficiently so that there is minimal deleterious effect on cells flowing in the channel.

Optical components, such as waveguide optics, may be integrated in processed layers of the substrate which are preferably fabricated in similar processes to those defining fluid channels. Such optical components may include waveguides for interrogation of the cell or support medium in the fluid channel. These may include evanescent field coupling.

which is interconnected, for example as shown in Figure 5. Channels 22 are fabricated in the device in order to direct the flow of fluid and cells. An electrode pair 16a and 16b is connected to a voltage supply (not shown) by way of a switch which is activated by the active device.

The electrode pairs 16a and 16b are preferably formed from gold plated pillars. It is the electrode pair which, when energised with a voltage pulse, form a discontinuity or weakness in the cell wall so as to permit the infusion of a substance. Optionally electrodes 16 perform the function of detection of the presence of a cell, by for example variations in capacitance between the electrodes 16a and 16b. It will be appreciated that other means for the detection of cells may be used, for example these may include an optical detector.

Fluid flowing through channel 22 permits the passage of cellular matter supported in the fluid through the electrode configuration. The fluid may pass along the surface of substrate 10 into a plurality of other channels (not shown) whence it is directed to a larger channel or capilliary tube for subsequent processing. Alternatively, as shown in Figure 3, channels may be formed through the substrate. In the embodiment shown in Figures 2 and 3, cells are located in well-like structure 15 and a potential difference is applied to each cell in parallel. The advantage of the embodiment shown in Figures 2 and 3 is that a very large number of cells may be transfected at substantially the same instant.

more pairs of electrodes 100, 120 are provided in contact with the channel, with electrical connections to external devices via tracks 140 and contacts 160. Electroporation potentials are provided by power supplies not shown. Alternatively, an electrode pair may be configured initially in a detection mode and when a cell is detected, switched into electroporation mode. A series of electrode pairs may be provided to give sequential potential treatments. The electrodes may also be used to measure properties of the cells.

As shown in Figure 4b the electrodes may use a common ground electrode 200 instead of discrete pairs. An electroporation electrode 240 shaped in order to concentrate the field towards the cell or a particular area of the cell. Detection electrodes 220 may be shaped for maximum sensitivity or simply planar. As in Figure 4c the channel 40 may be close to or even smaller than the diameter of the cells in all or part in order to give close contact between the electrodes and the cell wall as shown in Figure 4c.

In all the above embodiments the pairs of electrodes may be on the same side of the channel or opposite sides.

Figure 4d shows an embodiment in which cells 180 are flowed into flow channel 40 from a supply reservoir not shown, in a carrier medium without the transfection material; the device 2 has a junction 330 shown as a T with a second channel 332 for supply of the material 334 to be transfected. Electrodes 338a, b and optionally 340 and 342 are electroporation electrodes. Again these may be discrete pairs or electrodes with a common ground. Again, the electroporation power supply may be a single potential

by a force created by the pressure drop of the flow between channels 4 and 52, or if the cell is a tight fit to the well 50, by a static pressure difference between these channels. Alternatively, the cell may be held in place by physical force from a plunger or deformable section in the top wall 55 of channel 4. A pressure pulse applied to material 34 in channel 54 will then force 34 into the cell. The pulse may be applied for example by deformation of a membrane 60 on a chamber 56. The large ratio of area of 60 and the orifice of channel 54 will easily produce a large sudden pressure for injection.

Additional and/or optional electrodes 62, 64 may be provided to electroporate the cell membrane around the orifice of channel 54, reducing the need for a pressure pulse. Electrodes 62, 64 may also or alternatively be used to detect the presence of the cell in order to initiate or control the process. Electrodes may additionally or alternatively be placed in other positions such as on the sidewalls or around the top of the well 50, e.g. as shown in positions 66, 68, or in contact with the material 34 in order to achieve the above.

Figure 6 shows a further embodiment in which a sheath flow arrangement is used to prevent cells adhering to sidewalls and to control their behaviour using fluid flow. Cells are introduced to the device through a flow channel 100 which is joined by one or more further channels 102 to provide a sheathing laminar flow, the boudaries between the flows being shown diagrammatically at 104. Electrodes 106 with connections 108 then achieve electroporation. These electrodes may advantageously be shaped in order to concentrate field and / or localise poration. Further pairs of electrodes or other variants as described above may be incorporated. Material to be transfected 34 may be

pressurising means or any suitable mechanism for displacing fluid such as electrostrictive means or electro-osmosis. This signal varies the pressure applied by pump 52 to the fluid present in the apparatus. The reason the fluid pressure is varied is so that as more cells enter wells 15 less pressure is required in order to drive cells into remaining vacant wells. If the pressure was maintained, due to the increase in impedance as wells become occupied, the pressure on one side of the substrate would rise and this would tend to urge cells through the wells. Alternatively or in addition to a very fine mesh (not shown) or other restrictive means may be applied to the backface of the wells which restrictive means permits the flow of fluid there through but prevent passage of cells. At the end of a cycle the restrictive means may be moved or removed thus permitting the cells to pass through the wells and a fresh charge of cells to be introduced. Cells are stored in a reservoir 54 and are introduced via valve 56 which is under control of the microprocessor 50.

The method will now be described with general reference to all the Figures. A cell 18 passes into well 15 and its presence is detected by proximity detectors. A signal is sent to a counter in microprocessor 50. The counter increments and the value stored therein may be used to modify one or more system parameters, for example pumping pressure. Electroporation then occurs either on the individual cell or simultaneously with one or more other cells.

The invention has been described by way of examples only. It will be understood variation may be made to the examples without departing from the scope of the invention.

6. Apparatus according to any preceding claim formed on a semiconductor substrate.

7. Apparatus according to claim 6 wherein the semiconductor substrate includes silicon.

8. Apparatus according to any preceding claim wherein the means for introducing the substance into the object includes a syringe.

9. Apparatus according to any preceding claim including means for determining the state or condition of the object following transfection.

10. Apparatus according to claim 9, when dependent upon claim 7, wherein the means for determining the state or condition of the object includes an optical sensor and an infra-red source.

11. Apparatus according to any preceding claim including sorting means arranged to direct transfected objects to a first repository and non-transfected objects to a second repository.

12. A plurality of apparatuses, according to claim 1 arranged in an array.

Fig.1.

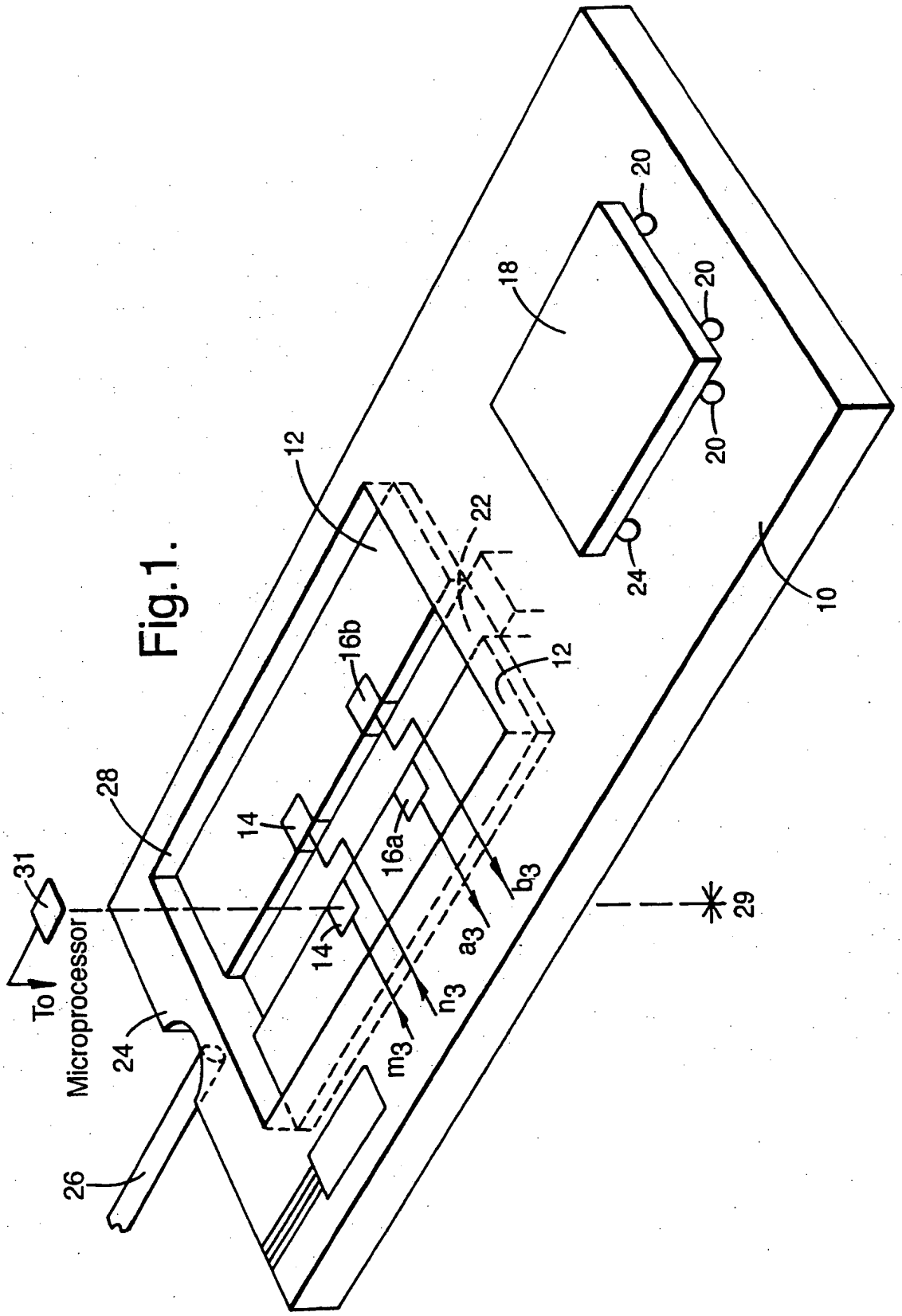


Fig.4a.

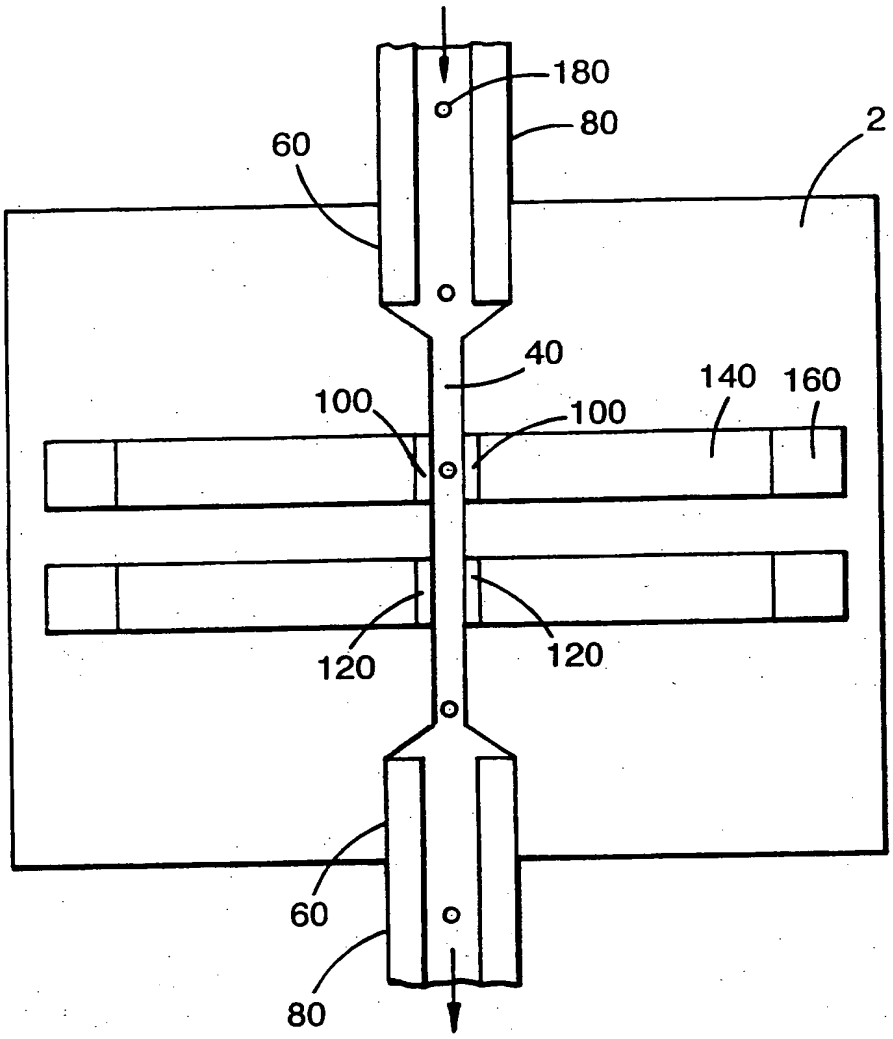



Fig.4b.

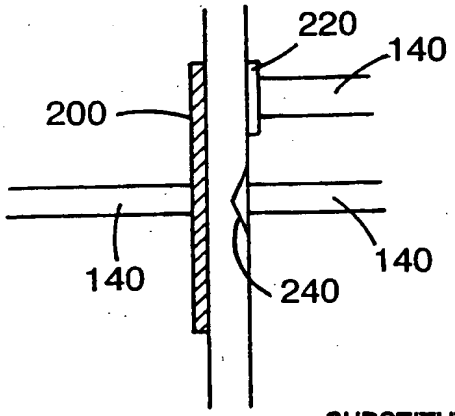



Fig.4c.

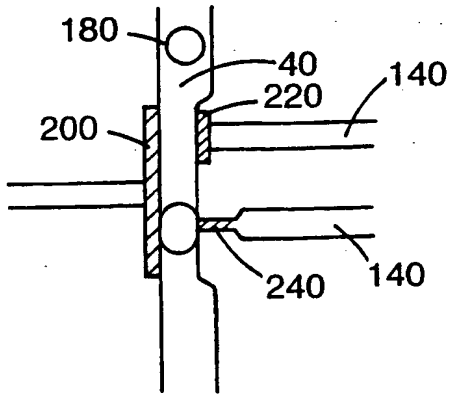


Fig.4e.

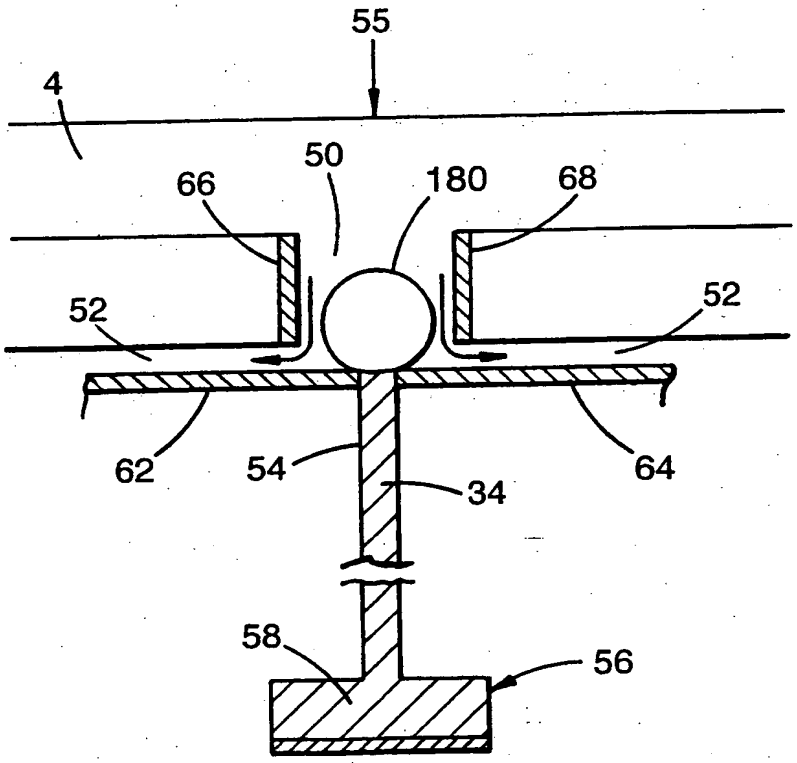
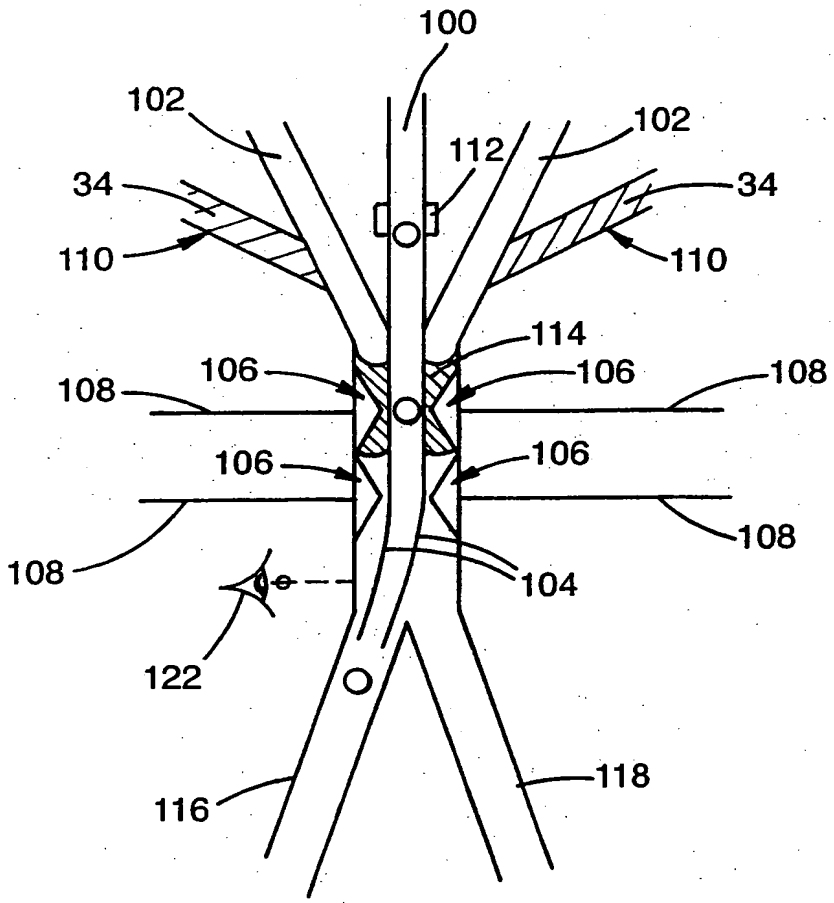


Fig.6.



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(74) Agent: **BROWN, Andrew, Stephen**; Astrazeneca, Global Intellectual Property, P.O. Box 272, Mereside, Aderley Park, Macclesfield, Cheshire SK10 4GR (GB).

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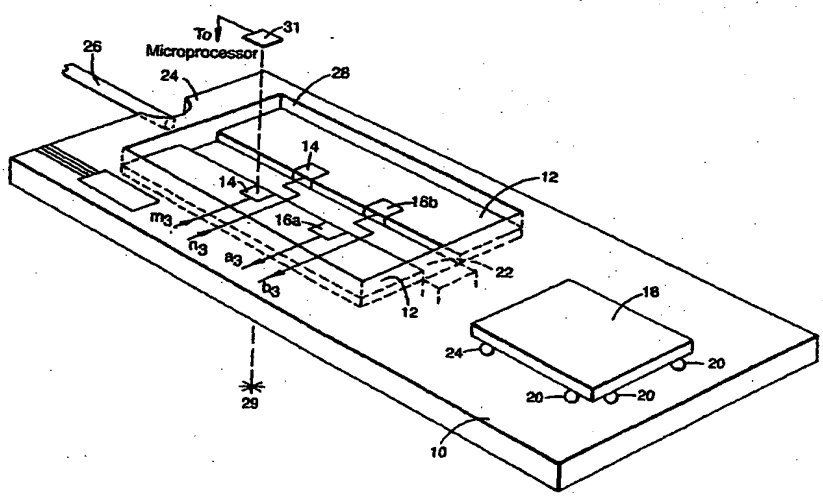
(54) Title: APPARATUS FOR, AND METHOD OF, INTRODUCING A SUBSTANCE INTO A CELL BY ELECTROPORATION




(57) Abstract: The invention relates to an apparatus for and method of introducing a substance into an object, particularly into a cell or cellular material. In a preferred arrangement apparatus receives the cell and preferably locates it between first and second electrodes and applies a voltage pulse to cause a disruption in the cell wall. This causes the cell to become permeable. The substance may then be introduced, for example under a fluid pressure. Cells may then be inspected and sorted into transfected and non-transfected types. This may be achieved automatically, for example by using electrophoresis.

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# INTERNATIONAL SEARCH REPORT

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 5 137 817 A (BUSTA, H.H. ET AL.)<br>11 August 1992 (1992-08-11) | 1,3,4,<br>6-8,12,<br>14 |
| A | abstract<br><br>page 1; figures 1,4A,4B<br>page 3; figures 4C,4E<br>page 5; figures 5-7<br>column 3, line 19 - line 29<br>column 3, line 41 -column 4, line 18<br>column 5, line 28 - line 46<br>column 6, line 39 - line 62<br>column 9, line 67 -column 11, line 31<br>column 12, line 13 - line 24<br>column 12 -column 14; claims<br>1,2,4,6-10,12,13,15 | 2,5,<br>9-11,13 |

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[X] Further documents are listed in the continuation of box C.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | US 4 894 343 A (TANAKA, S. ET AL.)<br>16 January 1990 (1990-01-16)<br>cited in the application<br>the whole document | 1-14 |

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